UPTAKE OF THREE PCB CONGENERS AND ENDOSULFAN BYDEVELOPING WHITE LEGHORN CHICKEN EMBRYOS (*GALLUS DOMESTICUS*)

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ABSTRACT

The uptake of three polychlorinated biphenyls (PCB congeners 105, 156, 189) and an organochlorine pesticide (endosulfan) by maternally exposed developing White leghorn chicken embryos (*Gallus domesticus*) was investigated. Artificially inseminated, adult hens were sub-cutaneously injected every four days with a mixture of the above chemicals resulting in the following concentrations: 126 µg/kg for PCB 105, 117 µg/kg for PCB 156, 110 µg/kg for PCB 189, and 178 µg/kg for endosulfan. Five eggs were removed from the incubator and dissected at 9, 14, and 19 days of incubation representing 43, 67 and 90% of development. The embryo, chorioallantoic membrane (CAM), and remaining egg contents (yolk/albumin) were separated from one another, weighed, and stored at -20ºC until analysis. Greater than 90% of the total mass of each chemical in the whole egg remained within the yolk/albumin through 14 days of development while 70% remained through 19 days of development. The yolk/albumin contained over 70% of the chemical mass within the egg at that stage of development even though it comprised only 20% of the total tissue mass within the egg. The embryo contained 17% to 30%, depending on the chemical, of the total mass within the egg at day 19 of development. The CAM contained 0.2% to 9% of the total chemical mass by day 19. While the proportion of PCB congeners in the embryo and CAM appeared to be inversely related to chlorination, no statistically significant differences were evident between congener proportions within a tissue. The majority of contaminants present within an egg remain external to the developing embryo through 90% of development.

INTRODUCTION

Discussion of avian embryo exposure to organochlorines centers on chemical presence within either the whole egg or yolk. Data has suggested that over 50% of organochlorine mass within the egg remains within the yolk at pipping and shortly post hatch (Custer et al. 1997, Pastor et al. 1996). Therefore, it is possible results gained from extraction and analysis of whole eggs or newly hatched chicks can result in overestimation of embryonic and chick exposure to organochlorines. What is unclear is just how much of the residual yolk is utilized by the newly hatched chick. If it is little utilized then maximum exposure from yolk absorption has been achieved at some point prior to hatch with the rest being excreted post hatch.

The purpose of this study was to investigate the timing of embryonic exposure to chemicals absorbed from the yolk and determine the proportion of the total egg chemical burden to which the embryo is exposed during development.

MATERIALS AND METHODS

- **Two hens were dosed sub-cutaneously every four days for approximately 3 weeks with 100µl of the following chemical mixture:**
  PCB congener 105 (1.71µg/µl)
  PCB congener 156 (1.59µg/µl)
  PCB congener 189 (1.50µg/µl)
  Endosulfan mixed isomers: a-isomer (1.84µg/µl), ß-isomer (0.58µg/µl)
- **Both hens were artificially inseminated following 1 week of injections.**
- **A total of 15 eggs were collected from the hens and incubated at approximately 95°F and 45% humidity.**
- **Five eggs were sacrificed at each of 9, 14, and 19 days of development. The embryo, CAM, and remaining egg contents (yolk/albumin) were separated from each other, weighed, and stored at –20°C for later extraction and analysis.**
- **Tissues were homogenized in $NaSO_4$, spiked with the recovery standard DCBP and Soxhlet extracted in 200ml dichloromethane for 20H. Extracts were concentrated by rotary evaporation, cleaned by gel permeation, and then silica gel chromatography. Extraction efficiencies for dosing compounds ranged from 92% to 106%. Recovery standard recoveries ranged from 78% to 128% (DCBP). A portion of the final extract volume was taken for gravimetric lipid determination. Extracts were analyzed by GC/ECD.**

Figure 1. PCB congener mass in tissue as a proportion of total congener mass in egg.

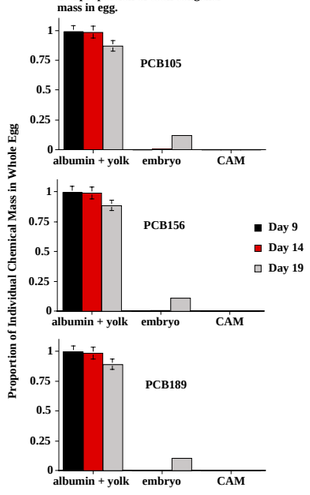


Figure 2. Endosulfan isomer mass in tissue as a proportion of total endosulfan mass in egg.

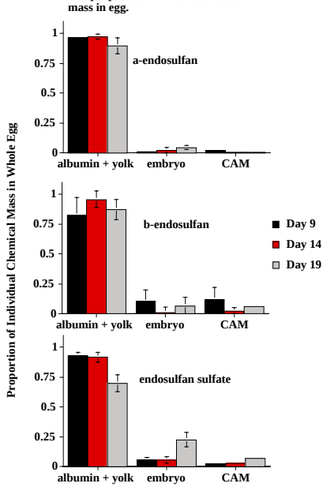


Figure 3. Tissue wet weight and lipid content at 9, 14, and 19 days of incubation.

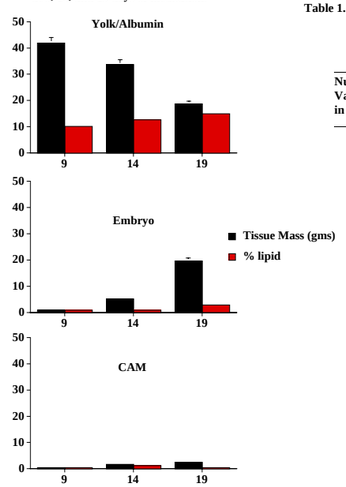


Table 1. Stepwise multiple regression analysis comparing the change in chemical mass within the embryo, asa proportion of total chemical mass in egg, with the change in both tissue weight (TSWT) and lipid content (%LIP) during development.

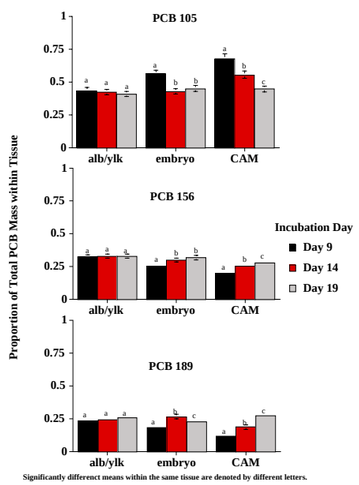
Number of Variables in Model*	R^2	Adj. R^2	C(p)	MSE	Variables
PCB 105					
1#	0.817	0.802	1.21	0.0001	TSWT
1	0.522	0.482	19.33	0.0004	%LIP
2	0.821	0.788	3.00	0.0001	%LIP TSWT
PCB 156					
1#	0.728	0.705	1.05	0.0001	TSWT
1	0.481	0.437	11.08	0.0002	%LIP
2	0.729	0.680	3.00	0.0001	%LIP TSWT
PCB 189					
1#	0.723	0.700	1.00	0.0001	TSWT
1	0.503	0.461	9.73	0.0001	%LIP
2	0.723	0.672	3.00	0.0001	%LIP TSWT
a-Endosulfan					
1#	0.358	0.305	1.05	0.1836	TSWT
1	0.220	0.155	3.44	0.2232	%LIP
2	0.361	0.245	3.00	0.1993	%LIP TSWT
b-Endosulfan					
1#	0.446	0.399	1.00	0.3983	%LIP
1	0.321	0.264	3.48	0.4879	TSWT
2	0.446	0.345	3.00	0.4343	%LIP TSWT
Endosulfan Sulfate					
1#	0.379	0.328	1.28	0.4138	TSWT
1	0.199	0.132	4.56	0.5340	%LIP
2	0.395	0.285	3.00	0.4401	%LIP TSWT

*** Significance level for inclusion into model was 0.15. All data were log transformed. # denotes significance at a = 0.05.**

RESULTS

- **The majority of PCB absorption into the embryo occurred during the later half of development (Fig. 1).**
- **Greater than 70% of PCB (Fig. 1) and endosulfan mass (Fig. 2) in the egg remained within the yolk/albumin.**
- **By day 19 of development, the embryo had absorbed no more than 20% of the total PCBs and 25% of the total endosulfan contained in the egg.**
- **Generally less than 10% of the organochlorine mass was absorbed by the embryo and transported into the CAM.**
- **PCB congener mass within the embryo, as a proportion of total congener mass within the egg, was significantly correlated (a=0.05) with tissue wet weight (Table 1).**
- **a-Endosulfan and Endosulfan Sulfate mass within the embryo was also significantly correlated with tissue wet weight.**
- **b-Endosulfan mass was more correlated with lipid content.**
- **Addition of the lipid content variable into a two variable model with tissue weight did not dramatically increase the R^2 above that of the model of tissue weight alone.**
- **The change in chemical mass within the CAM was not significantly correlated with either tissue weight or lipid content.**
- **Tissue wet weight and lipid content changes in the embryo, yolk/albumin, and CAM are shown in Figure 3.**
- **PCB congener profile within both the embryo and the CAM changed during development (Fig. 4). The proportion of total PCB mass in both the embryo and CAM comprised by PCB105 decreased during development relative to PCB156 and PCB189 proportions, which increased during development.**
- **The proportion for all three PCB congeners in the yolk/albumin remained statistically the same relative to one another during development (Fig. 4).**
- **The proportion of neither of the endosulfan isomers nor the sulfate metabolite changed significantly during development (Fig. 5).**
- **Lipid weight based PCB concentrations increased with developmental age in the yolk/albumin whereas it did not change in either the embryo or CAM (Table 2). Tissue weight based PCB concentrations increased in both the yolk/albumin and embryo but not in the CAM. Endosulfan concentrations were more variable relative to embryo age with no clear trend evident.**

Figure 4. PCB congener mass in tissue as a proportion of total PCB mass in tissue.



Significantly differenct means within the same tissue are denoted by different letters.

Figure 5. Endosulfan isomer mass in tissue as proportion of total Endosulfan mass in tissue.

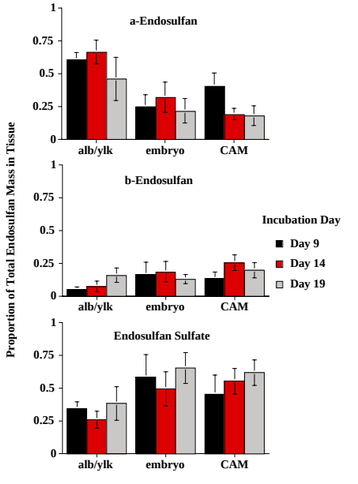


Table 2. Chemical concentrations in embryonic tissues at 9, 14, and 19 days of development.

Tissue Concentrations, ng/gm wet weight (standard error)

Incubation Day	Tissue	a-Endosulfan	b-Endosulfan	Endosulfan sulfate	PCB 105	PCB 156	PCB 189
9	Albumin + Yolk	4.20 (1.271)	0.30 (0.018)	2.20 (1.532)	57.14 (6.740)	42.39 (5.534)	31.23 (4.594)
9	Embryo	0.17 (2.196)	0.13 (0.036)	3.61 (0.437)	7.12 (0.415)	3.17 (0.163)	2.30 (0.155)
9	CAM	0.42 (0.980)	0.35 (0.002)	2.94 (0.418)	11.45 (1.767)	3.34 (0.659)	1.97 (0.341)
14	Albumin + Yolk	3.58 (0.335)	0.10 (0.116)	1.41 (0.863)	145 80 (38.435)	110.98 (29.520)	80.94 (20.306)
14	Embryo	0.02 (0.054)	0.08 (0.050)	0.47 (0.550)	7.77 (1.675)	5.60 (1.395)	4.89 (1.140)
14	CAM	0.06 (0.033)	0.02 (0.003)	0.46 (0.473)	7.11 (1.320)	3.34 (0.733)	2.61 (0.709)
19	Albumin + Yolk	1.05 (0.466)	0.42 (0.251)	1.69 (1.272)	293.08 (72.870)	227.68 (50.604)	177.13 (36.534)
19	Embryo	0.10 (0.071)	0.27 (0.179)	0.75 (0.454)	30.36 (7.187)	21.49 (5.605)	15.47 (3.992)
19	CAM	0.11 (0.089)	0.06 (0.050)	1.72 (1.105)	5.90 (0.943)	3.66 (0.628)	3.62 (0.651)

Tissue Concentrations, ng/gm lipid (standard error)

9	Albumin+Yolk	39.38 (10.092)	2.81 (0.033)	20.22 (13.539)	547.65 (57.251)	405.47 (45.797)	298.26 (38.298)
9	Embryo	77.48 (62.729)	12.51 (2.686)	618.97 (181.717)	585.47 (37.154)	270.82 (24.601)	297.85 (37.020)
9	CAM	264.51 (246.243)	58.14 (17.990)	465.73 (78.822)	2019.99 (276.128)	595.83 (105.396)	334.51 (13.500)
14	Albumin+Yolk	29.02 (2.973)	0.84 (0.924)	11.35 (6.819)	1149.65 (307.826)	875.62 (237.157)	638.83 (163.215)
14	Embryo	11.49 (7.668)	6.40 (5.680)	249.04 (302.739)	1020.58 (347.040)	682.70 (195.218)	589.22 (158.772)
14	CAM	9.80 (6.123)	8.54 (8.684)	19.68 (6.789)	1115.51 (310.003)	530.81 (156.000)	404.09 (129.242)
19	Albumin+Yolk	7.26 (3.197)	2.98 (1.810)	11.45 (8.086)	1981.88 (417.034)	1543.06 (289.594)	1202.55 (209.90)
19	Embryo	6.50 (6.526)	1.98 (1.166)	42.09 (35.859)	1353.48 (662.671)	960.40 (477.651)	675.76 (321.19)
19	CAM	25.40 (21.428)	61.28 (43.201)	386.99 (258.051)	1251.50 (189.870)	778.98 (133.859)	772.83 (143.580)

DISCUSSION

- **In this study, greater than 70% of the organochlorine contaminants in the egg remained in the yolk/albumin through at least 19 days of embryonic development (Fig. 1). This was one day prior to pipping and one to two days before hatch. These data agree with previous studies. Residual yolk sacs in 1-day old double-crested cormorant chicks (*Phalacrocorax auritus*) contained approximately 60% of total PCBs (Custer et al. 1997) while over 54% of organochlorines in pipping audouin's sea gulls (*Larus audouinii*) were in the residual yolk sacs (Pastor et al. 1996). However, it is not clear as to whether or not the residual yolk is completely utilized or if at least some of it is excreted not allowing complete absorption of organochlorine chemicals. Both scenarios would lead to a decrease in organochlorine burden in the yolk sac relative to the burden in the entire egg or chick.**
- **PCB absorption from the yolk into the embryo most closely followed embryonic growth (Table 1). Regression analyses indicated that between 72% and 82% of the variability among incubation days was explained by variation in embryo weight. Variation among incubation days with lipid content was not significant enough (a < 0.15) to be included in the regression models. Lipid content inclusion in a two variable model did not significantly change the coefficients of determination. Even though the lipid content data were insignificant, they were reported here for completeness.**
- **Neither variation in the CAM's tissue mass nor lipid content explained the changes in PCB mass in the CAM among development days (Table 1). Previous research unsuccessfully attempted to show that lipid content might explain organochlorine content in the CAM (Pastor et al. 1996). The data from this study also shows that lipid content does not adequately explain organochlorine content in the CAM.**
- **The pattern of PCB absorption from the yolk by the embryo and distribution once within the embryo may differ among homologue groups. The proportion of PCB105 within the embryo relative to the proportion of both PCB156 and PCB189 decreased with developmental age (Fig. 4). The pattern was similar for the CAM. Although the characteristics of different congeners within the same homologue group are not likely to be identical, data from this study point out the influence of differing chemical structure on PCB absorption from the yolk and distribution within the embryo.**

CONCLUSIONS

- **The majority of organochlorines within an egg remain external to the developing embryo. Therefore, the embryo is not exposed to the entire chemical burden contained in the egg.**
- **Absorption of PCBs from the yolk by the embryo is most related to embryonic growth. Embryonic exposure will increase with age.**
- **Chlorination influences absorption of PCBs from the yolk resulting in embryonic exposure to chemical profiles different from what would be predicted based on extraction and analyses of whole eggs.**

REFERENCES

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ACKNOWLEDGEMENTS

E.I. Du Pont de Nemours and Company graciously provided funding for this study.

The authors would like to thank the following individuals for their assistance during this study: Milton Taylor, James Dancik, Katheryn Bryant, Dr. Glenn Birrenkott, Janet Bouknight, Frances Harper, Virginia Waldrop, Joey, and Margie Peden-Adams.

The authors would also like to thank the National Ocean Service and NIST for use of their analytical equipment for sample extraction and analysis.